600-1-081CONCIP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Steinman et al. EXAMINER: Schwadron, Ronald B.

SERIAL NO.: 09/925,284 ART UNIT: 1644

FILED: August 9, 2001

FOR: ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, MICHEL NUSSENZWEIG, hereby declare and state that:

1. I am a Howard Hughes Investigator, Sherman Fairchild Professor and Senior Physician at Rockefeller University having received my Ph.D. degree from the Rockefeller University in 1981 and my M.D. degree from New York University in 1982. I received postdoctoral medical and scientific training at Harvard University. My full curriculum vitae is attached hereto as Exhibit A.

2. My principal area of research is in Immunology and among other positions I serve as reviewer in numerous funding agencies of many countries, including the National Institute of Health, March of Dimes, Dana Foundation. I also have served as reviewer for numerous scientific journals, and I am the Editor of the Journal of Experimental Medicine and the Journal of Immunologic Methods.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ENHANCED ANTIGEN DELIVERY AND MODULATION OF THE IMMUNE RESPONSE THEREFROM", having U.S. Serial Number 09/925,284, which is a continuation-in-part of U.S. application Serial Number 09/586,704, filed on June 5, 2000, which is a continuation of U.S. Serial Number 08/381,528, filed on January 31, 1995, now abandoned.

4. I have reviewed the disclosure of the present application, with particular emphasis on the support in the application as filed for the preparation and generation of antibodies against human and mouse DEC-205 proteins.

5. The present application claims a method of enhancing the development of tolerance to a pre-selected antigen for which tolerance is desired in a mammal comprising exposing ex vivo or in vivo dendritic cells from said mammal to a conjugate comprising said pre-selected antigen covalently bound to an anti-human DEC-205 antibody or an anti-murine DEC-205 antibody that binds to human DEC-205 under conditions that promote dendritic cell quiescence. More particularly, the pre-selected antigen is selected from the group consisting of allergens, autoantigens and antigens participating in allograft rejection. The human DEC-205 protein has a carboxy terminal and amino terminal amino acid sequence as disclosed in the parent application, U.S. Serial Number 09/586,704, filed on June 5, 2000, as SEQ ID NOs.: 1 and 2, respectively. Furthermore, as noted in the parent application USSN 09/586,704, the first 19 amino acid residues of the amino terminal human DEC-205 protein (designated as SEQ ID NO: 13 in the parent application) were used to generate antibodies that reacted with human DEC-205. The sequences from the parent application have now been included in the sequence listing for the present application and are designated as SEQ ID NO: 7 for the carboxy terminal, SEQ ID NO: 8 for the amino terminal, and SEQ ID NO: 9 for the first 19 amino acids of the amino terminal used for antibody generation.

6. The subject matter of the present application was based on work performed in my laboratory, whereby the human DEC-205 molecule was cloned and expressed (Guo, M., Gong, S., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., Nussenzweig, M.C. & Steinman, R.; (2000), A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells, *Human Immunology* 61:729-738). Anti-human DEC-205 antibodies were then prepared by immunizing animals with the first 19 amino acid residues from the N terminal fragment of the cloned human DEC-205 protein.

7. To summarize briefly, the cloning of human DEC-205 was done through use of a cDNA fragment of the 3' portion of mouse DEC-205. This was used to screen a human lymphocyte and thymus cDNA library using standard procedures known to those skilled in the art. In particular, the cDNA fragment of mouse DEC-205 was used to screen a human lymphocyte matchmaker cDNA library (EBV-transformed human peripheral blood B lymphocytes) and a human thymus 5'-stretch plus cDNA library in a Ogt10 vector (Clontech Laboratories, Palo Alto, CA, USA). Positive clones were identified by DNA sequencing on

both strands using Sequenase (United State Biochemical, Cleveland, OH, USA), or the dye determinator kit (PE Applied Biosystems, Foster City, CA, USA) and automated sequencing (Applied Biosystems model 371). The human cDNAs were expressed in pEF-BOS modified to carry a 3' human Fc fragment that was in frame with the insert. DEC-205 leader, CR domain, and FnII domains were amplified from plasmids by PCR using 5' MG31 primers and 3' MG35 primers. The 5' -- primer contains a SpeI site, while the 3' – primer contains a NotI site and codes for PRR at the junction point of DEC-205 and the Fc tag. The human DEC-205 Fc fusion protein was produced by transiently transfecting 293 cells using calcium phosphate mediated gene transfer. The fusion protein was purified on protein A sepharose and was then used to inject mice. Following several booster injections, the serum was tested for antibodies that reacted with the CR-FnII domain of the human DEC-205 molecule using Western blot procedures. Afterwards, the spleens were harvested from those animals showing a positive reaction and were fused with Sl'2/0 cells. The supernatants were screened by ELISA, dot blot, thymus tissue staining and FACS analysis. Cell clones that secreted anti-human DEC-205 antibodies were further subcloned and expanded.

8. The present application teaches methods for inducing tolerance by conjugating an antigen to a DEC-205 antibody for targeting to the DEC-205 receptor on specific cells, such as dendritic cells, under conditions that promote dendritic cell quiescence. The antibodies that react with the DEC-205 proteins, in particular, the anti-human DEC-205 antibodies, were prepared using the first 19 amino acid residues from the amino terminal end of the cloned human DEC-205 protein, as described in the parent application U.S. Serial Number 09/586,704, and further attested to in this declaration. Thus, it is my belief that the disclosure of the present application provides sufficient written description for a person skilled in the art to prepare such antibodies that react with human DEC-205 protein as presently claimed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: 1/12/05

Michel Nussenzweig, M.D., Ph.D.

EXHIBIT A

CURRICULUM VITAE

Name: Michel C. Nussenzweig

Date of Birth: February 10, 1955

Education:

1975	B.A. - New York University College of Arts and Sciences
1981	Ph.D. - The Rockefeller University
1982	M.D. - New York University School of Medicine

Clinical Training:

1982-1985	Intern & Resident, Internal Medicine Massachusetts General Hospital
1984-1985	Clinical Fellow, Infectious Diseases Massachusetts General Hospital

Postdoctoral Training:

1986-1989	Harvard Medical School, Department of Genetics

Professional Appointments

1990-1996	Assistant & Associate Professor, The Rockefeller University
1990-1999	Assistant & Associate Investigator, Howard Hughes Medical Institute
1996-present	Professor & Senior Physician, The Rockefeller University
1999-present	Investigator, Howard Hughes Medical Institute
2000-present	Sherman Fairchild Professor of Immunology, The Rockefeller Univ.

Honors & Awards

Summa Cum Laude, New York University College of Arts and Sciences - 1975; Phi Beta Kappa, New York University College of Arts and Sciences - 1975; Alpha Omega Alpha, New York University Medical School - 1982; Bertram M. Gresner Memorial Research Award, New York University School of Medicine – 1982; Elected Member American Society of Clinical Investigators - 1997, Solomon A. Berson Award for Basic Science - 2002

Teaching:

Immunology, Course Organizer

Institutional:

Chair, Transgenic Facility Coordinating Committee
Chair, Animal Care and Use Committee

Chair, Hospital Seminar Committee
Member, Immunology Search Committee
Member, Institutional Review Board for Biohazards, Radioisotopes, Toxic Chemicals, and Carcinogens
Member Hospital GCRC Scientific Advisory Committee
Elected Senior Faculty Representative Academic Council
Member, Virology Search Committee

National
Arthritis Foundation Molecular Immunology study section 1993-1996
NIH Immunobiology Study Section Ad Hoc reviewer 1998, and 1999
NIH ALY Study Section Ad Hoc Reviewer, 1999
NIH NIAID Council Ad Hoc 1998
Organizer Keystone Symposium on Dendritic Cells 1998
Organizer Keystone Symposium on B Cells 1999
March of Dimes Review Committee 1999-
External Reviewer LMGD NICHD 2000
Damon Runyon Cancer Research Fund Review Committee 2000-2002
American Association of Immunologists Program Committee 2000-
NIH ALY Study Section Member 2001-
Organizer Keystone Symposium on B Cell Biology 2003

Editorial:

1996-Present	Editor, The Journal of Experimental Medicine
1999-Present	Editor, The Journal of Immunological Methods
2000-Present	Transmitting Editor, International Immunology
2002-Present	Advisory Editor, Nature Reviews Immunology

Consultant:
Abgenix, Fremont, CA
Zycos, Lexington MA

Professional Memberships:
American Association of Immunologists
American Medical Association
The New York Academy of Sciences
Kunkel Society
Harvey Society

Publications:

1. Steinman, R.M., Witmer, M.D., **Nussenzweig, M.C.**, Chen, L.L. & Cohn, Z.A. Dendritic Cells: an important new cell type in the mixed leukocyte reaction. In: Kaplan JG, ed. Proc. of the 13th International Leukocyte Culture conference. *Elsevier/North Holland Publ.* Co. 273. (1979)

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3. **Nussenzweig, M.C.** & Steinman, R.M. Contribution of dendritic cells to stimulation of the syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196-1212. (1980)

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6. **Nussenzweig, M.C.**, Steinman, R.M., Unkeless, J.C., Witmer, M., Gutchinov, B., & Cohn, Z.A. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J. Exp. Med.* 154:168-187. (1981)

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56. Yu, W., Misulovin, Z., Suh, H., Hardy, R.R., Jankovic, M., Yannoutsos, N & **Nussenzweig, M.C.** Coordinate regulation of RAG1 and RAG2 by cell type-specific DNA elements 5' of RAG2. *Science* 285:1080-1084 (1999)

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Immunological Reviews 1993, No. 136
Printed in Denmark

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Immunological Reviews
ISSN 0105-2896

New Immunosuppressants: Testing and Development in Animal Models and the Clinic: with Special Reference to DSG

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INTRODUCTION

Since the start of clinical transplantation in the late '50s and early '60s, the development of immunosuppressive regimens has taken two main directions, the first of which has involved the search for new immunosuppressive drugs. Experiments, mainly in rodents and dogs, have been used to discover and test new immunosuppressive drugs. The drugs azathioprine, cyclosporine A, FK506, and RS61443 have all reached the clinic because of being effective as monotherapy in experimental animals. Secondly, in conjunction with their introduction into the clinic, the drugs mentioned above have mostly been used either in combination with steroids or in various combinations one with another. Their efficacy in monotherapy has been well established but the scientific support for using these drugs in combinations has sometimes been rather weak (Starzl et al. 1963, Klintmalm et al. 1981). This is easy to understand, as the requirements demanded of clinical studies aimed at developing new immunosuppressive drugs have increased, mainly because the results are far better than in the '60s and '70s. Thus, studies require many more patients per study group to test a new immunosuppressive drug today than they did 10 years ago when cyclosporine (CyA, European multicentre trial group 1982 and Canadian multicentre study group 1985) was introduced.

The first part of this presentation will discuss the possibilities for testing new immunosuppressive drugs in a rodent model, and further to test optimal drug

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combinations in animal experiments for the future clinical use of the newly developed immunosuppressive drugs. Our group's developmental work on deoxyspergualin (DSG) will serve as a model to describe the potentials and limitations of this type of experimental testing. Secondly, rodent xenograft models will be discussed with reference to the possibility of producing immunosuppressive drugs to inhibit antibody formation. And, finally, some reason for work with other immunosuppressive drugs will be described in our model.

Testing new drugs in animal models

Some of the immunosuppressive drugs currently used in clinical organ transplantation have developed during *in vitro* testing, particularly those providing inhibition of mitogen and mixed leukocyte responses. However, several of the new substances are either ineffective *in vitro* or lipophilic to an extent where dose-response patterns are difficult to interpret. Therefore, *in vitro* testing is not always sufficient in developing new immunosuppressive drugs.

The most widely used technique to test immunosuppressants for organ transplantation has been heterotopic cardiac grafting in rodents, particular in rats (Ono & Lindsey 1969, Heron 1974). These transplantations are technically easy to perform and, in experienced hands, reproducible. *A main problem in the rodent model for organ allografting is the ease with which rejection is usually suppressed.* Thus rodents usually accept organs without evidence of rejection with daily medication of immunosuppressants given as monotherapy. The doses required are often somewhat lower than those used in the human being. Furthermore, the treatment can often be discontinued after 2-4 weeks and the grafts will be permanently accepted, at least in certain strain combinations (White et al. 1982, Mjörnstedt et al. 1984).

The mechanism behind this permanent graft survival after a short course of treatment is not always absolutely clear; however, in the majority of cases it seems that an active antigen-specific suppressive mechanism is developed. Thus, the new graft from the same strain can be given, but a third-party graft is rejected.

A further problem, in addition to absence of rejection episodes on drug treatment and mechanisms for induction of tolerance, seems to be a low level of antibody formation against the graft. Thus, a second graft after immunization with the first graft is rarely hyperacutely rejected, mainly because of the absence of antibodies causing hyperacute rejection. This is in contrast to the human situation where immunization leads to antibody formation and a clear risk of a subsequent graft with similar HLA being hyperacutely rejected.

Testing of multiple-drug regimens

In clinical practice monotherapy immunosuppression is fairly rare. In practice, only CyA (Calne et al. 1979) is currently used as monotherapy in certain trans-

plant centers. However, the majority of transplant centers use cyclosporin in combination with steroids (double-therapy) or steroids plus azathioprine (triple therapy). Many transplant centers also use monoclonal or polyclonal anti T-cell or antilymphocyte preparations as induction therapy (quadruple-therapy). The scientific support for the use of these regimens is rather weak. Even large randomized studies often fail to show improved results with multiple-drug therapy. However, many clinicians have experience telling them that multiple-drug regimens are better, especially when they handle difficult cases, as in regrafting, when the first graft has failed due to rejection. The possibility in large randomized studies for testing new drug combinations for these difficult cases is almost non existent for various reasons, e.g. the number of centers that must be involved and the cost. Therefore simple animal experimental support to give evidence of superiority of certain drug combinations could be the best way to select new ways to combine drugs in a fruitful manner in order to improve overall transplant results and especially to improve results in the difficult cases where first-line therapy has already failed.

The testing of drugs in the clinic setting for maintenance of immunosuppression has been largely empirical, but some support has also been provided from randomized studies. This is rarely the case for treatment of rejection. Here, almost all the clinical rational treatment of rejection is based on empirical findings. The only available small animal model has to produce rejection having been given no immunosuppression and treatment has to start just prior to complete rejection of the organ. Animal experimental data supporting clinical maneuvers are therefore scarce. Except for the OKT-3 trial (Orthe Multicenter Transplant study group, 1985) very few randomized studies have been carried out comparing different forms of antirejection treatment in the clinic.

From the above discussion it is evident that animal experiments to support modes of clinical action are warranted. However, today's small animal model seem to be insufficient to produce data for clinical decision-making. Large animal models are, for various reasons, difficult to handle. Thus many of the immunosuppressive drugs have side effects in dogs and monkeys other than those seen in humans. In particular, dogs have difficulty surviving doses of immunosuppression they would need to be given for reasonable efficacy. Experiments in monkeys are, in addition, even more costly and not generally amenable to test all hypotheses. After this airing of problems in the clinical field it is clear that small animal models are sought. The rest of this review will concentrate on our effort to develop such models and our experience with using these.

LS2616 as an inducer of rejection in rats treated with Cyclosporin

LS2616 (Linomide®, Kabi Pharmacia, Uppsala Sweden) is an immunostimulator (Ståhlandske et al. 1982) with several effects. The drug is only effective *in vi*

and not *in vitro* and therefore is not that well-characterized. However, LS2616 is known to stimulate both B-cell. (Spetzberg-Hagher & Larsson-Sjöard 1989) T-cell (Larsson et al. 1989) and natural killer (Kalland et al. 1985) cell-mediated immune reactions. Our first experience with this substance was an experiment in which we tried to potentiate the immunosuppressive effect of cyclosporine with what we thought was a possible immunosuppressive effect of LS2616 on the survival of heterotopic rat heart allografts. Somewhat to our surprise, animals given LS2616 rejected the heart allografts at the same tempo as non-treated animals. Cyclosporin offers no protection whatsoever to the survival of the grafts (Wauters et al. 1988, see also Table II).

The unique point about this finding was that rejection took place in this rodent model despite seemingly adequate baseline immunosuppression. The dose and the administration of the drug seem to be rather uncritical. Doses from 10 mg/kg per day to 160 mg/kg per day of LS2616 seem to yield more or less the same result. The drug was easily administered in the drinking water. With one or two exceptions, in 5 years the model has never failed to produce rejection in the presence of cyclosporin A as immunosuppressant. Several rat strain combinations

TABLE I

Effect of various immunosuppressants on heterotopic cardiac graft survival in the absence or presence of LS2616 (160 mg/kg b.w.)

Group nr.	Strain	Immuno-suppression	Dose	Time	LS	Survival days	n
1	PVG-Wky	none				8	12
2	PVG-Wky	CyA	10 mg	0-9		19	5
3	PVG-Wky	CyA	10 mg	0-9	+	8	15
4	PVG-Wky	none			+	8	9
5	DA-Lew-Lew	none				8	15
6	DA-Lew-Lew	CyA	10 mg	0-9		22	7
7	DA-Lew-Lew	CyA	10 mg	0-9	+	8	5
8	PVG-Wky	pred	15 mg	0-stop		18	8
9	PVG-Wky	pred	15 mg	0-stop	+	9	6
10	PVG-DA	none				9	9
11	PVG-DA	ATG	0.02	0		18*	10
12	PVG-DA	ATG	0.02	0	+	9	9
13	PVG-DA	none			+	8	8
14	PVG-Wky	DSG	2 mg	0-9		18	9
15	PVG-Wky	DSG	2 mg	0-9	+	13	10
16	PVG-Wky	DSG + CyA	2 mg	0-9	+	40	11
17	PVG-Wky	FK	0.3 mg	0-9	-	24	8
18	PVG-Wky	FK	0.3 mg	0-9	+	18	10
19	PVG-Wky	MC1288	0.1 μg	0-9		22	8
20	PVG-Wky	MC1288	0.1 μg	0-9	+	11	11

* 10% permanent survival. CyA given 0-stop.

have been used and it seems to work in every strain combination, except wi Brown Norway as donors (an only 2-month-old result which is presently bei explored). The timing of the therapy also seems to be fairly non-critical. T addition of LS2616 to the recipient either several days prior to transplantatio or a few days after transplantation did not alter the time of rejection, i.e. remained at approximately 9 days. Pretreatment of the donor did not change t result.

The dose of cyclosporine was also not very critical. Doses sufficient to maintu graft survival up to sublethal doses were all totally reversed by LS2616.

Administration of higher doses of cyclosporine to a DA-to-PVG combinati normally produces around 50% permanent graft survivors. The mechanism behi this permanent graft survival has been shown to be tolerance (Nagao et al. 198 Administration of LS2616 totally abrogates the induction of tolerance. In the experiments LS2616 was given to the animals after cyclosporine therapy (Wauch et al. 1991). However, it is noteworthy that already-induced stable tolerance, i animals carrying a graft permanently accepted some 60–80 days after transpla tation, is not lost if LS2616 is given (Table II).

One of the first questions we raised was whether this was due to some sort pharmacologic interaction with cyclosporine. It seems not to be; thus, wh steroids were used instead of cyclosporine, exactly the same results were obtain (Table I, Cerdin 1989). Secondly, cyclosporine trough levels were sufficient suggest that it was not due to pharmacological interaction.

Morphological and immune histochemical analyses of the grafts showed th the same cellular events take place as in untreated animals undergoing rejectio

We also used this treatment in kidney transplants, because class I-disparo rat strains do normally reject kidney grafts. LS2616 failed to induce rejection these animals.

Based on the above-mentioned observations, we drew the conclusion th LS2616 induces rejection in CyA- or in steroid protected grafts by a rejectio mechanism not grossly different from that in non-immunosuppressed anima rejecting their grafts. The specific stimulatory effect of LS2616 seems to be

TABLE II

Ability of LS2616 to inhibit tolerance after heterotopic cardiac transplantation

Animals	CyA-dose	Time	LS	Time (start of LS)	Tolerant animal (> 100 days)
PVG-DA	20 mg	0-14			13/27
PVG-DA	20 mg	0-14	+	14-stop	0/4
PVG-DA	20 mg	0-14	+	40-stop	5/5
PVG-DA	20 mg	0-14	+	60-stop	5/5
PVG-DA	20 mg	0-14	+	90-stop	3/3*

* > 200 days.

allow the expansion of the effector face of the rejection mechanism (Wanders et al. 1991).

Our current understanding of the phenomena induced by LS2616 remains largely the same. Today we have no evidence to suggest that LS2616 in this model produces any other cellular effect.

At this time we also drew the tentative conclusion that this model could be used to explore and test the immunosuppressive effect of some of the new immunosuppressants. This is still our working hypothesis. The authors of this paper have realized that the number of combinations with the new immunosuppressants is almost infinite. Therefore we have joined forces to evaluate the most likely combinations to be used in clinical practice within the next few years.

Other immunosuppressive drugs tested by LS2616

The first substance that we came to test in this model was DSG (Yoshikawa et al. 1988) DSG had been shown to function as an immunosuppressant in various *in vivo* experimental models. The effect *in vitro* was, however, questioned. Our first practical encounter with DSG was in a xenograft model where it prolonged graft survival (Gannedahl et al. 1990). Today it is well known that DSG inhibits T-cell activity (Todo et al. 1988) and B-cell activity (Fujii et al. 1990) whereas the effect on macrophages and other antigen-presenting cells is debated. The immunosuppressive effect of DSG was dependent on the dose. The immunosuppressive effect of 2 mg of DSG was largely reversible by LS2616, but higher doses of DSG were not affected (Gannedahl et al. 1993a). We encountered a further problem which has limited our ability to explore how DSG acts, and that is that in practically all other situations it gives rather large variability in graft survival times. It is also of interest that the doses of DSG that are effective in the rodent model are similar to those used clinically (Ohlman et al. 1992, Gannedahl et al. 1992a).

FK506, introduced by the Pittsburg group, has proven to be a valuable clinical immunosuppressant especially in clinical liver transplantation (Starzl et al. 1988). The effect of this drug is also affected by LS2616 treatment (Table I). The effect is, however, not complete in the doses used here. Further ongoing experiments will tell us whether the pattern is similar to that of DSG, namely that in lower but still therapeutic doses the drug effect is better reversed. This will be interesting since the mode of action of CyA and FK506 are thought to be the same. A tentative but far from proven explanation is that some cytokines inhibited by CyA are better inhibited by FK506 and in turn are otherwise used by the LS2616-driven rejection process.

Antithymocyte globulin (ATG) has long been used in the clinical setting as induction therapy. Careful titration of the ATG dose will produce improved but not permanent graft survival in a rat model. The results displayed in Table I show that the effect of a single low dose of ATG can be reduced by LS2616. ATG, FK506 and DSG treatment produce similar results with regard to LS2616 despite the fact that they all are considered to have different modes of action. Ongoing experiments will hopefully clarify whether these similarities exist also at other doses. This is important because, if the pattern is similar in different dose ranges, it points to common pathways of the rejection mechanism or the immunological network interaction of these immunosuppressants offering graft protection and tolerance.

A potential new era for the development of immunosuppressants is vitamin D. Because of the effects on calcium levels its use is limited (Rigby 1988). However analogues without serum calcium effects can be immunosuppressive (Hinder et al. 1991). In Table I we have included the effect of such an analogue, MC1288 on graft survival in the presence or absence of LS2616. The results are exactly comparable to those of CyA. Future testing will let us know whether this is a novel drug action that has any place in the immunosuppressive arsenal of the transplant surgeon or physician.

Drug combination's additive-synergistic immunosuppressive effect

As mentioned several times already in this paper, the clinical application of immunosuppressive drugs has mainly been in combination therapy. The practical rationale behind multiple-drug therapy is to increase the total immunosuppressive effect by adding several drugs and by not using one drug at too high a dose, thus avoiding some of the side effects of the drug. The similarities between this reasoning and the same type of reasoning when combination therapy is used to reduce blood pressure in hypertensive patients is obvious. Whereas the efficacy of combinations of antihypertensive drugs and reduction of side effects seem feasible to measure in large studies, it has proven very difficult either by registry work or by randomized studies to, for example, prove the value of triple-therapy versus double-therapy (Brinker et al. 1990, Koutte et al. 1988, Penticell et al. 1988, Lindholm et al. 1992). From a clinical standpoint it would therefore be of great value if new immunosuppressive drugs introduced into the market could be provided with a test for additive or synergistic immunosuppressive effects in animal models.

We attempted to do exactly this with DSG. Table I shows the result of continuous administration of cyclosporine and LS to rats receiving a heterotopic cardiac allograft. Animals had also been given "induction therapy" (initial therapy) with DSG for the first 9 days in a dose of 2 mg/kg. As can be seen, the CyA therapy by itself had no effect and DSG therapy by itself had very little effect in the presence of LS. When the two drugs are given together, DSG as part of the induction therapy greatly improved the results obtained. The immunosuppressive effect obtained by giving these two drugs seems to bear the require

ments for synergism. Thus we are describing the synergistic effect of the two immunosuppressive drugs. This result has been published only recent (Cannedahl et al. 1993a) but will hopefully prompt pharmaceutical companies to test the clinical value of induction therapy with DSG.

Hopefully this type of experiment may also serve as a model to research future immunosuppressive drugs when they are going to be introduced clinically; i.e., whether they display additive or synergetic effects with CyA. Such an experimental selection procedure may somewhat reduce the otherwise almost infinite number of clinical trials needed to place a new immunosuppressive drug on the therapeutic market.

The above conclusion is, of course, only valid if the model in itself is entirely valid for the clinical situation. Until now we have had no data to suggest otherwise. It is tempting to speculate that LS induces cytokine release which induces rejection as described for IL-2 addition (Heidecke et al. 1984). However, in the long run it would be necessary to know if the effect of LS2616 is analogous to the rejection process occurring on this seemingly adequate CyA therapy. From a pragmatic point of view we have also, for the moment, nothing better to offer, and at least the procedure circumvents the problem of easy tolerance induction and absence of rejection in low levels of immunosuppressive therapy.

Xenografts as a model for anti B-cell immunosuppressive effects

One final aspect of the problem of testing immunosuppressants in small animal models has been that rodents seem to reject allografts with very low or absent B-cell responses. After immunization with cells or graft, a new graft survives 3–5 days (Haltunen et al. 1987). Even LS2616 treatment produces animals which do not reject their second graft hyperacutely. That is, animals immunized with one allograft, when grafted 6 weeks later reject the second allograft at an accelerated tempo but not hyperacutely. That is, the graft rejects within 3 days. This would probably not be the situation in humans where preformed antibodies to MHC components will lead to hyperacute rejection of a second graft.

We initially found that DSG prolonged the survival of a first xenograft in a mouse-to-rat combination which is a fairly distant concordant xenograft model. (Concordant is defined as transfer of a xenograft to another species where detectable amounts of hemagglutinating antibodies are not present at the time of grafting; for review see Auchincloss 1989). Rats sensitized with the first mouse xenograft reject a second mouse xenograft (Cannedahl et al. 1992b) at the tempo of a severe hyperacute rejection, namely less than a minute. This rejection is accompanied by the presence of hemagglutinating antibodies, increased amounts of lymphocytotoxic antibodies and presence of an antibody reacting to tissue components of the heart graft as determined by immunofluorescence. Rats given DSG for 3 weeks at a dose of 5 or 10 mg/kg bodyweight do not reject the second

xenograft hyperacutely. Instead the second xenograft will survive for a few hours. These animals, thus treated, do not develop the antibodies detected above (Cannedahl 1993b). Thus in this experimental model it seems likely that we are testing for a desirable effect to add to the therapeutic arsenal in allografting, namely that of an anti B-cell drug. As mentioned in the introduction, rejection of a human allograft may be accompanied by the formation of antibodies.

CONCLUDING REMARKS

In the future, it would seem to be reasonable to test new immunosuppressive drugs, here exemplified by DSG, in allograft models not only to show prolonged allograft survival but also to define the drug's behavior in the presence of LS2616. Moreover, one may find it possible to test new triple or quadruple or other regimens including CyA in this LS model for detection of synergy/additive effect. B-cell immunosuppressant properties may be tested in closely related concordant xenograft models.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council, The Magn and Birger Gustavssons fund, the Gelin fund and the University of Gothenbur

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THE JOURNAL OF IMMUNOLOGY

BRIEF REVIEWS

Of Mice and Not Men: Differences between Mouse and Human Immunology

Javier Mestas and Christopher C. W. Hughes[1]

Mice are the experimental tool of choice for the majority of immunologists and the study of their immune responses has yielded tremendous insight into the workings of the human immune system. However, as 65 million years of evolution might suggest, there are significant differences. Here we outline known discrepancies in both innate and adaptive immunity, including: balance of leukocyte subsets, defensins, Toll receptors, inducible NO synthase, the NK inhibitory receptor families Ly49 and KIR, FcR, Ig subsets, the B cell (BLNK, Btk, and λ5) and T cell (ZAP70 and common γ-chain) signaling pathway components, Thy-1, γδ T cells, cytokines and cytokine receptors, Th1/Th2 differentiation, costimulatory molecule expression and function, Ag-presenting function of endothelial cells, and chemokine and chemokine receptor expression. We also provide examples, such as multiple sclerosis and delayed-type hypersensitivity, where complex multicomponent processes differ. Such differences should be taken into account when using mice as preclinical models of human disease. The Journal of Immunology, 2004, 172: 2731–2738.

Mice are the mainstay of in vivo immunological experimentation and in many respects they mirror human biology remarkably well. This conservation of function is reflected in recent reports on the sequencing of both the human and mice genomes, which reveal that to date only 300 or so genes appear to be unique to one species or the other (1). Despite this conservation there exist significant differences between mice and humans in immune system development, activation, and response to challenge, in both the innate and adaptive arms. Such differences should not be surprising as the two species diverged somewhere between 65 and 75 million years ago, differ hugely in both size and lifespan, and have evolved in quite different ecological niches where widely different pathogenic challenges need to be met—after all, most of us do not live with our heads a half-inch off the ground. However, because there are so many parallels there has been a tendency to ignore differences and in many cases, perhaps, make the assumption that what is true in mice—in vivo veritas—is necessarily true in humans. By making such assumptions we run the risk of overlooking aspects of human immunology that do not occur, or cannot be modeled, in mice. Included in this subset will be differences that may preclude a successful preclinical trial in mice becoming a successful clinical trial in human.

In this review our aim is not to suggest that the mouse is an invalid model system for human biology. Clearly, with so many paradigms that translate well between the species, and with the relative ease with which mice can now be genetically manipulated, mouse models will continue to provide important information for many years to come. Rather, our aim is to sound a word of caution. As therapies for human diseases become ever more sophisticated and specifically targeted, it becomes increasingly important to understand the potential limitations of extrapolating data from mice to humans. The literature is littered with examples of therapies that work well in mice but fail to provide similar efficacy in humans (2–7). By focusing on some known differences between mouse and human immunology we hope to spur interest in this area and encourage others to note differences where they occur.

Structure and general characteristics

The overall structure of the immune system in mice and humans is quite similar. As this topic has been recently reviewed in depth (8), we will not go into great detail here. One difference worth noting is that whereas mice have significant bronchus-associated lymphoid tissue, this is largely absent in healthy humans (9), possibly reflecting a higher breathable Ag load for animals living so much closer to the ground.

The balance of lymphocytes and neutrophils in adult animals is quite different: human blood is neutrophil rich (50–70% neutrophils, 30–50% lymphocytes) whereas mouse blood has a strong preponderance of lymphocytes (75–90% lymphocytes, 10–25% neutrophils) (10). It is not clear what, if any, functional consequence this shift toward neutrophil-rich blood in humans has had.

Tyrosine kinase receptor expression on putative hemopoietic stem cells (HSC)[2] shows a reciprocal pattern, with mouse HSC being predominantly c-*kit*^{high}, *flt-3*$^{-}$, whereas human HSC are predominantly c-*kit*^{low}, *flt-3*$^{+}$ (11).

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Received for publication November 12, 2003. Accepted for publication January 23, 2004.

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[2] Abbreviations used in this paper: HSC, hemopoietic stem cells; iNOS, inducible NO synthase; γ_c, common γ-chain; DETC, dendritic epidermal T cells; MS, multiple sclerosis; DTH, delayed-type hypersensitivity; EC, endothelial cells.

0022-1767/04/$02.00

Innate immunity

One of the first lines of defense in higher organisms, and often the only defense in lower animals, is the growing family of antimicrobial peptides, and in particular the defensins. These are important in mucosal defense in the gut and in epithelial defense in skin and elsewhere (12, 13). Neutrophils are a rich source of leukocyte defensins in humans, but defensins are not expressed by neutrophils in mice (14). In contrast, Paneth cells, which are present in the crypts of the small intestine, express >20 defensins (cryptdins) in mice but only two in human, likely reflecting different evolutionary pressures related to microorganism exposure through food intake. There are also differences in processing of defensins (Table I).

The last few years have seen a renewed focus on the field of innate immunology, spurred in large part by identification of the Toll-like family of receptors—the TLRs (15). This field is still relatively young and so far a limited number of differences have been noted between mice and humans (Table I).

There has been considerable controversy as to whether human macrophages express NO. Expression of functional inducible NO synthase (iNOS; NOS2) in mouse macrophages has been clearly demonstrated and iNOS mRNA is readily induced by IFN-γ and LPS (16). However, these same inflammatory mediators have failed to show consistent effects on human macrophages, hence the confusion. Recent work suggests that other mediators, such as IFN-$\alpha\beta$, IL-4 plus anti-CD23, and various chemokines, are actually far more efficient in inducing iNOS in human macrophages (17). However, the controversy is not dead yet (18).

Using different strains of mice a susceptibility locus for CMV infection, *cmv1*, was identified and later shown to encode the Ly49 family of proteins (19). There are at least 14 members and most are expressed on NK and NKT cells, where the majority act as NK inhibitory receptors for MHC I molecules. The Ly49 family is absent in humans, who use the KIR family as NK inhibitory receptors (20). KIR proteins are highly diverged from the Ly49 family and have Ig rather than C-type lectin domains in their extracellular domain; however, similarly to Ly49 they also recognize MHC class I. The ligands for mouse and human NKG2D differ: in humans, NKG2D binds the polymorphic MHC class I-like molecules MHC-I chain-related A, MHC-I chain-related B, and the UL16 binding protein family, whereas in mouse NKG2D binds to H-60 and Rae1β. The significance of these differences to CMV infection and to NK biology in general have not been determined.

Adaptive immunity

FcR represent a link between the adaptive immune system, which generates Ab, and the innate immune system, which can respond to Ab-Ag complexes through capture by FcR expressed on macrophages, neutrophils, eosinophils, mast cells, and dendritic cells. There are several differences in FcR expression between mice and humans. In humans, FcαRI (CD89) is an important IgA receptor expressed by neutrophils, eosinophils, monocytes/macrophages, dendritic cells, and Kupffer cells (21). Mice lack FcαRI and presumably use alternative receptors, such as Fcα/μR, the transferrin receptor (CD71) and polymeric IgR, which also binds IgM. Humans also express two IgG receptors not found in mice: FcγRIIA and FcγRIIC are closely related single-chain FcR, each of which has a single ITAM motif in the intracellular domain. In contrast, most other FcR associate with ITAM-containing signal transduction subunits (22).

In addition to differences in FcR there are well-known differences in expression of Ig isotypes between mice and humans, and direct correlations between subtypes within classes in each species are hard to make. Mice make IgA, IgD, IgE, IgM, and four subtypes of IgG: IgG1, IgG2a, IgG2b, and IgG3. Interestingly, in the C57BL/6, C57BL/10, SJL, and NOD strains of mice there is no expression of IgG2a, instead these mice express the novel IgG2c (23). Humans in contrast express two subtypes of IgA—IgA1 and IgA2—along with single forms of IgD, IgE, and IgM. In humans there are also four subtypes of IgG: IgG1, IgG2, IgG3, and IgG4; however, these are not direct homologues of the mouse proteins. While different subtypes have differing abilities to bind FcR or fix complement, the differences between mice and humans are not considered significant. In contrast, there are differences in class switching: in mice, IL-4 induces IgG1 and IgE, whereas in humans, IL-4 induces switching to IgG4 and IgE. In contrast, IL-13 has no effect on mouse B cells but induces switching to IgE in humans (24).

There are some interesting differences in B cell development that relate to the roles of several signaling molecules. BLNK (Src homology-2 domain containing leukocyte-specific phosphoprotein-65) is an adapter protein that is rapidly phosphorylated by Syk after cross-linking of the B cell Ag receptor. It then serves as a scaffold for downstream signaling components such as Grb2, Vav, Nck, and PLC-γ. B cell development in mice lacking BLNK is blocked at the pro-B to pre-B transition, resulting in low numbers of IgM^+ B cells, but no mature $IgM^{low}IgD^{high}$ B cells, appearing in the periphery (25). A naturally occurring mutation in the human BLNK protein has been identified that results in a splicing defect preventing protein expression. In this patient there was also a block in the pro-B to pre-B transition; however, there was also a complete absence of B cells in the periphery, suggesting a more severe block in human B cell development than in mice (26).

Similarly discrepant phenotypes have been noted in mice lacking functional BCR-associated tyrosine kinase Btk (27) and in mice lacking λ5 (28), the L chain component of the pre-BCR (Table I). Differences in mature B cells between mice and humans were recently reviewed (29), and include mutually exclusive expression of CD5 and CD23 on mouse but not human B cell subsets, and CD38 expression on human, but not mouse, plasma cells.

The discrepant phenotypes discussed above for BLNK, Btk, and λ5 should be treated with some caution as the human diseases usually arise due to mutations in the relevant genes rather than deletions of whole exons as seen in the mouse knockout models. In some cases, however, identical mutations have been found, or created, in mice and the discrepant phenotype remains. This is the case for human XLA and mouse XID, which both involve Btk (30, 31).

The development and regulation of T cells also differs between mice and humans. Thy-1 is a GPI-linked Ig superfamily molecule of unknown function. It is expressed on thymocytes and peripheral T cells in mice and has been widely used as a T cell marker in the thymus. In humans, however, it is only expressed on neurons. The basis of this tissue specificity is suggested to be the presence or absence of an Ets-1 binding site in the third intron of the gene (32).

Table I. *Summary of some known immunological differences between mouse and human*

	Mouse	Human	Notes	Refs.
Hemotopoiesis in spleen	Active into adulthood	Ends before birth		
Presence of BALT	Significant	Largely absent in healthy tissue		9
Neutrophils in periph. blood	10–25%	50–70%		10
Lymphocytes in periph. blood	75–90%	30–50%		10
Hemotopoietic stem cells	c-*kit*^{high}, *flt-3*$^{-}$	c-*kit*^{low}, *flt-3*$^{+}$		11
TLR2 expression on PBL	Low (induced on many cells including T cells)	Constitutive (but not on T cells)	Binds lipopeptides	88
TLR3	Expressed on DC, Mac. Induced by LPS	Expressed by DC. No LPS induction	Binds dsRNA	88, 89
TLR9	Expressed on all myeloid cells, plasmacytoid DC and B cells	Expressed only on B cells, plasmacytoid DC and N	Binds CpG	90, 91
TLR10	Pseudogene	Widely expressed		
Sialic acid Neu5GC expression	Widespread	Absent	Binds pathogens	92
CD33	Expressed on granulocytes	Expressed on monocytes	Binds sialic acids	93
Leukocyte defensins	Absent	Present	neutrophils	14
Paneth cell defensins	Processed by MMP7. Stored pre-processed	Stored as pro-form. Processed by trypsin		94, 95
Paneth cell defensins	At least 20	Two		13
Macrophage NO	Induced by IFN-γ and LPS	Induced by IFN-α/β, IL-4^{+} anti-CD23		17
CD4 on macrophages	Absent	Present		96
Predominant T cells in skin and mucosa	γ/δ TCR (dendritic epidermal T cells—DETC)	α/β TCR		40
γ/δ T cells respond to phospho-antigens	No	Yes		97
CD1 genes	CD1d	CD1a,b,c,d		41
NK inhibitory Rs for MHC 1	Ly49 family (except Ly49D and H)	KIR		20
NKG2D ligands	H-60, Rae1β	MIC A, MIC B, ULBP	NK activating Rs	98
fMLP receptor affinity	Low	High		99
FcαRI	Absent	Present		21
FcγRIIA, C	Absent	Present		22
Serum IgA	Mostly polymeric	Mostly monomeric		21
Ig classes	IgA, IgD, IgE, IgG1, IgG2a*, IgG2b, IgG3, IgM * absent in C57BL/6, /10, SJL and NOD mice, which have IgG2c	IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM		23
Ig CDR-H3 region	Shorter, less diverse	Longer, more diverse		100
BLNK deficiency	IgM^{high} B cells in periphery	No peripheral B cells		25, 26
Btk deficiency	Normal pre-B and immature B	Blocks pro-B to pre-B transition		28
λ5 deficiency	"leaky" block at pro-B to pre-B transition	Blocks pro-B to pre-B transition		28
CD38 expression on B cells	Low on GC B cells, off in plasma cells	High on GC B cells and plasma cells		29
B cell CD5 and CD23 expression	Mutually exclusive	Co-expression		29
IL-13 effect on B cells	None	Induces switch to IgE		24
Thy 1 expression	Thymocytes, peripheral T cells	Absent from all T cells, expressed on neurons		32
Effect of γ_c deficiency	Loss of T, NK, and B cells	Loss of T, NK, but B cell numbers normal		33, 34
Effect of Jak3 deficiency	Phenocopies γ_c deficiency	Phenocopies γ_c deficiency		31
Effect of IL-7R deficiency	Blocks T and B cell development	Only blocks T cell development		35, 36
ZAP70 deficiency	No CD4^{+} or CD8^{+} T cells	No CD8^{+} T but many nonfunctional CD4^{+}	Related to *syk* level?	37, 38
Caspase 8 deficiency	Embryonic lethal	Viable—immunodeficiency		62, 63
Caspase 10	Absent	Present		62
IFN-α promotes Th1 differentiation	No	Yes	Mutant stat2 in mice	44
Th expression of IL-10	Th2	Th1 and Th2		51
IL-4 and IFN-γ expression by cultured Th	Either/or	Sometimes both		
CD28 expression on T cells	On 100% of CD4^{+} and CD8^{+}	On 80% of CD4^{+}, 50% of CD8^{+}		54
ICOS deficiency	Normal B cell numbers and function, normal IgM levels	B cells immature and severely reduced in number, low IgM	Possibly age-related	55–57
B7-H3 effects on T cells	Inhibits activation	Promotes activation		101–2
ICAM3	Absent	Present	DC-SIGN ligand	103–4
P-selectin promoter	Activated by TNF and LPS	Unresponsive to inflammation		58
GlyCAM	Present	Absent		105
MHC II expression on T cells	Absent	Present		59–61
Kv1.3 K^{+} channel on T cells	Absent	Present	Regulates Ca flux	64, 65
MUC1 on T cells	Absent	Present	Regulates migration?	106
Granulysin	Absent	Present	In CTL	43

(*Table continues*)

Table I. *Continues*

	Mouse	Human	Notes	Refs.
CXCR1	Absent	Present		66, 67
IL-8, NAP-2, ITAC, MCP-4, HCC-1, HCC-2, MPIF-1, PARC, eotaxin-2/3	Absent	Present	Chemokines	66, 67
MRP-1/2, lungkine, MCP-5	Present	Absent	Chemokines	66, 67
IFN-γ effects in demyelinating disease	Protective in EAE	Exacerbates MS		4, 69–70
DTH lesions	Neutrophil-rich	Lymphocyte-rich		73, 74
Constitutive MHC II on EC	Absent	Present		80
EC present Ag to CD4+ T	No	Yes	Memory T only	75–77
CD58 (LFA-3)	Absent	Present	CD2 ligand	82
T cell dependence on CD2-ligand interactions	Low	High		82
CD2-ligand interaction	Lower affinity, with CD48	Higher affinity, with CD58		82
CD40 on EC	Absent	Present		83, 84
Vascularized grafts tolerogenic?	Yes	No		5
Microchimerism induces graft tolerance?	High success rate	Low success (expts. in non-human primates)		7
Passenger leukocytes	Account for graft immunogenicity	Do not account for graft immunogenicity		6

Similar to the development of B cells, mutation of key signaling molecules in T cells has markedly different effects in mice and humans. Several cytokine receptors, including those for IL-2, IL-4, IL-7, IL-9, and IL-15, share a common signaling chain called common γ chain (γ_c). Perhaps not surprisingly, deletion or mutation of this gene, which is on the X chromosome, results in severe immunological defects. Interestingly, these differ between human and mouse XSCID (33, 34). Numerous mutations have been identified in the human γ_c gene that inhibit function, and in most of these cases the result is a dramatic decrease in the number of T cells and NK cells. However, B cell development is normal, although function is impaired, likely due to the lack of T cell help. In marked contrast, B cell numbers are greatly diminished in γ_c-null mice. Given that IL-7R deficiency in mice blocks both T and B cell development (35), but only blocks T cell development in humans (36), it is likely that B cell development in humans is independent of IL-7. The major signal transducer for γ_c is JAK3 and mutation of this gene phenocopies the γ_c mutation in both mice and humans; that is, a lack of T and NK cells in human with the addition of a severe B cell defect in mice (31).

Interesting differences have also been noted in ZAP70-deficient mice and humans. ZAP70 is essential for TCR signaling in both developing and mature T cells, and compromised signaling results in SCID. In humans the defect results in normal numbers of $CD4^+$ T cells and absent $CD8^+$ T cells. However, the $CD4^+$ T cells are nonfunctional. In contrast, an identical mutation introduced into the mouse ZAP70 results in a block in differentiation of both T cell subsets at the double-positive stage (37). It has been suggested that the "leakiness" of the human mutant is due to incomplete down-regulation of the protein tyrosine kinase Syk in human thymocytes, compared with mouse thymocytes (38).

The study of γ/δ T cells has revealed a number of significant differences between mice and humans. T cells expressing γ/δ TCR are found in all organisms that have α/β receptors and yet their function is still largely an enigma (39). Mouse skin contains a large fraction of cells bearing a TCR encoded by a single Vγ and Vδ gene. These Vγ5-Vδ1 T cells appear to be oligoclonal, reside in the epidermis, and are known as dendritic epidermal T cells (DETC). DETC represent the predominant T cell in mouse skin, whereas cells bearing α/β receptors predominate in human skin and are found mostly in the dermis. Indeed, a cell with DETC characteristics has not been identified in humans (40). Human but not mouse γ/δ T cells have been suggested to recognize Ag presented by CD1 molecules—in particular CD1b (41). Interestingly, of the five CD1 molecules found in humans (designated CD1a, b, c, d, and e), only CD1d is expressed in mice (41). Similarly to γ/δ T cells the CD1 family of molecules has been implicated in the pathogenesis of tuberculosis, but their precise role has yet to be defined (42, 43). The differing expression of CD1 genes between mice and humans may well turn out to impact activation of both α/β and γ/δ T cells in tuberculosis, as both subsets can recognize a variety of Ags presented by CD1 molecules.

An often critical component of adaptive immunity is the skewing of T cell differentiation toward Th1 or Th2 phenotypes and this process represents another area of interaction between the innate and adaptive arms of immunity. In humans, the type I IFN, IFN-α, is secreted by several cell types in response to viral infection, including macrophages, and acts on T cells to induce Th1 development. This process is dependent upon STAT4 activation, and its recruitment to the IFN-α receptor by STAT2. In mice, however, IFN-α fails to induce Th1 cells and does not activate STAT4 (44).

The existence of polarized T cell populations was first demonstrated by Mosmann and colleagues (45) and since then has become a guiding principle for T cell activation. While polarization is relatively easy to observe in mice the paradigm has never been as clear-cut in the human system. Th1 and Th2 cells can certainly be found in human disease (46, 47); however, there is a growing recognition that in many diseases clear distinctions cannot be made and that T cells of both persuasions can often be generated simultaneously (48–50). For example, in mice, IL-10 is considered to be a Th2 cytokine, whereas in humans both Th1 and Th2 cells can make IL-10 (51). The response of mice and humans to schistosomiasis is remarkably different. Epidemiological data suggest that a Th2 response involving eosinophils and IgE may be key to combating infection in humans (52), whereas in mice effector cell activation by IFN-γ, a Th1 response, is essential for clearance of the parasite (53).

To become fully activated T cells require both a primary, Ag-dependent signal, and a second, Ag-independent or costimulatory signal. One of the best characterized costimulatory receptors is CD28, which is expressed by close to 100% of mouse $CD4^+$ and $CD8^+$ T cells. In contrast, only 80% of human $CD4^+$ and 50% of human $CD8^+$ T cells express CD28 (54), perhaps accounting for the remarkable efficacy of CTLA-4Ig in blocking T cell activation in mice. It will be interesting to see if expression of the CD28-related costimulatory molecule ICOS segregates with $CD28^-$ T cells in humans. The recent report on the identification of a human ICOS deficiency pointed to a further difference between costimulation in mice and humans. Whereas in mice the loss of ICOS does not affect either the number of mature B cells, their maturation status or their secretion of IgM (55, 56), the loss of ICOS in humans results in a severe reduction in B cell number, maturation status and secretion of IgM (57). Given the critical role of T cell CD40L in T-B interactions it would be interesting to know what the level of CD40L expression was on this patient's T cells and whether expression of this molecule is dependent upon ICOS signaling in humans. Two novel members of the B7 family of costimulatory molecules, B7-H3 and DC-SIGN, have recently also been suggested to have different roles in mice and humans (Table I).

P-selectin is constitutively expressed by endothelial cells (EC) and mediates leukocyte rolling by interactions with specific sugar residues carried by mucins. Interestingly, murine P-selectin can be strongly up-regulated by inflammatory mediators such as TNF and LPS, whereas the human gene is nonresponsive (58). It is interesting to speculate as to whether E-selectin in humans, which is strongly up-regulated by TNF, is the more important selectin on human EC for mediating leukocyte rolling.

Once activated, human T cells express MHC class II molecules whereas murine T cells do not. It has been suggested that human T cells can capture, process, and present Ag and that they express B7 and may therefore help to amplify an ongoing immune response (59, 60). In contrast, Ag presentation by T cells may also promote T cell anergy (61) or activation-induced cell death. It is not clear why this function is nonessential in mice, but it is an attractive hypothesis that it may relate to T cell homeostasis and the requirement in humans for maintaining, in a limited compartment, a greater diversity of memory T cells for a considerably longer period of time than is required in mice. T cell homeostasis requires programmed cell death (apoptosis) of unwanted cells. Caspase 8 and caspase 10 are downstream of death receptors in humans and overlap in some of their functions (62). Mice lack caspase 10 and the deletion of caspase 8 is embryonic lethal. Lack of caspase 8 in humans results in immunodeficiency, suggesting a role for this effector in lymphocyte activation as well as death (63). Greater redundancy in death receptor regulators in humans may relate to the longer lifespan and associated increased risk of developing cancer.

A critical step in activation of a T cell is the generation of a sustained calcium flux. In human T cells the inward flow of calcium ions is balance by an outward flow of K^+, mediated in large part by the Kv1.3 K^+ channel. Inhibitors of this channel very specifically block T cell activation in vitro and are being pursued as novel immunosuppressive agents (64). However, in vivo evidence to support such a function is missing as mouse T cells do not express this channel (65).

The movement of immune cells into and through tissues is coordinated by a huge array of chemokines and chemokine receptors and, not surprisingly, differences have emerged between the murine and human systems. While it is still too early to say definitively what such differences may mean, as there appears to be considerable redundancy built into the system, it is worth noting what is currently known. CXCR1 is present in humans but not in mice (66). The chemokines IL-8 (CXCL8), neutrophil-activating peptide-2 (CXCL7), IFN-inducible T cell α-chemoattractant (CXCL11), monocyte chemoattractant protein (MCP)-4 (CCL13), HCC-1 (CCL14), hemofiltrate CC chemokines-2 (CCL15), pulmonary and activation-regulated chemokine (CCL18), myeloid progenitor inhibitory factor-1 (CCL23), and eotaxin-2/3 (CCL24/CCL26) have all been identified in humans but not in mice. Conversely, CCL6, CCL9, lungkine (CXCL15), and MCP-5 (CCL12) have been identified in mice but not humans (66, 67).

Differences in immune system biology

Multiple sclerosis (MS) provides a fine example of both differences and similarities between mouse and human immunology. MS is a multifactorial disease that appears to have a large autoimmune component (68). Experimental autoimmune (allergic) encephalomyelitis is a widely used model for MS that mimics the demyelination seen in central and peripheral nerves in MS. Several studies have indicated that IFN-γ is protective in experimental autoimmune (allergic) encephalomyelitis as neutralizing Abs exacerbate disease, potentially by blocking induction/activation of suppressor activity (69, 70). It was surprising, therefore that clinical trials were not successful; indeed they were stopped because treatment with IFN-γ was found to exacerbate disease (4). In contrast, studies in mice suggested that blocking VLA-4 ($\alpha_4\beta_1$ integrin)-VCAM-1 interaction might help in MS (71) and this has indeed carried through successfully into human trials (72). These studies highlight how caution is required when extrapolating results from mouse studies to the clinic, but suggest that mouse models can successfully predict some therapies for human disease.

An interesting difference exists in the appearance of delayed-type hypersensitivity (DTH) reactions in mice and humans. In humans, around four hours after Ag challenge neutrophils can be seen forming a "cuff" around the venules. This is followed by a dramatic influx of mononuclear cells, such that by 24–48 h the lesion is mostly mononuclear with a mix of T cells and macrophages (73). Paradoxically, in mice where the peripheral blood has a relative paucity of neutrophils compared with humans, the DTH response tends to be more neutrophil rich (74). In addition, elicitation of murine DTH requires much higher concentrations of Ag than in humans.

There is now considerable evidence that human EC can present Ag to resting memory $CD4^+$ and $CD8^+$ T cells (75–77), whereas in mice, $CD8^+$ T cells can be activated by EC (78), but $CD4^+$ T cells cannot (B. Rosengard, personal communiction). As $CD4^+$ T cell-mediated activation of macrophages is thought to drive human DTH responses the suggestion has arisen that in humans, Ag transport to lymph nodes by Langerhans cells may not be necessary as EC may trigger the recall response at the site of challenge. A teleological argument can be made for the need to present Ag locally in humans but not necessarily in mice. It has been estimated that once a cell enters the lymphatics in humans it takes ~24 h to return to the

circulation if it is not retained in a node (79). Based on the higher cardiac output of mice as a proportion of their total blood volume compared with humans (5–10 ml/min, 2 ml total volume in mice; 5 L/min, 5 liter total volume in humans) it is reasonable to suppose that return of lymph is at least as fast in mice as it is in humans. Then it becomes a matter of scale. We calculate that an Ag traveling from toe to an inguinal lymph node in the groin should take ~12 h in humans and 20 min in mice. As the human DTH response begins around 4 h after secondary Ag challenge, it is possible that triggering of recall responses may occur by different mechanisms in mice and humans, involving draining of Ag to lymph nodes in mice, compared with local Ag presentation in humans.

Both human and mouse EC express MHC class I. Most human EC in vivo also constitutively express MHC class II molecules, whereas mouse EC do not (80). Thus, human EC can present Ag to $CD4^+$ T cells, as well as to $CD8^+$ T cells. A major costimulatory molecule on human EC is CD58 (LFA-3), a ligand for CD2 (81). Mice do not have the gene for CD58, which arose by CD2 gene duplication after the two lineages split. In mice the CD2 ligand is CD48; however, the distribution of this molecule differs from that of CD58 in humans, and the two-dimensional affinity for the mouse CD2-CD48 interaction is 40- to 50-fold lower than that for human CD2-CD58 interactions (82). In addition, gene deletion and Ab blocking studies have shown that mouse T cell activation is much less dependent on CD2 interactions than is the case for human T cells. Human EC also express CD40 and the ICOS ligand GL-50, whereas murine EC do not (83, 84).

The Ag presenting ability of human EC may have significant consequences for transplantation. For example, in many rodent models vascularized grafts are tolerizing, whereas such grafts are rapidly rejected in humans (5). Numerous studies have shown that purging mouse tissues of $CD45^+$ cells before transplantation dramatically extends the life of the graft, sometimes even inducing tolerance. In sharp contrast, purging human tissues of $CD45^+$ cells provides no benefit as the grafts are still rapidly rejected (6). In addition, the establishment of microchimerism in mice has been quite successful in inducing tolerance, whereas this has not been the case in humans (7). The implication of these findings is that there are major differences between mice and humans in their responses to grafted tissue, and that this may relate to the Ag-presenting ability of human, but not murine, EC.

Natural selection and the immune system

Most, if not all, of the differences we have noted between mouse and human immunology have likely become fixed during the 65 million years since our divergence because they provide some selective advantage. In all likelihood these adaptations are in response to new pathologic challenges from microorganisms, which have very short generation times and often have high mutation rates (85). In consequence, mammalian MHC molecules and NK cell inhibitory receptors have also evolved rapidly (9, 86). It should also be noted that some changes may be fixed primarily as a result of the nonimmune role of that gene—reiterative use of genes is a well recognized phenomenon during development, a good example being the important nonimmunological role of VCAM in chorioallantoic fusion and placentation (87). Thus, both the immune system as a whole, and some of its individual components (B and T cell repertoires) are shaped by natural selection.

Mice evolved in a quite different environment to humans and have been exposed to different Ags and their immune systems might therefore be expected to have evolved in subtly different ways. Mice not only live in different ecological niches, they are also much smaller and have significantly shorter lifespans. These are not trivial differences—as noted above, leukocyte transit times may be quite different in mice and humans, and a larger, broader repertoire of B and T cells must be maintained for many years in humans (up to 50 mouse lifetimes). Thus many changes may be to accommodate increased size of the organism, to regulate larger and more diverse pools of Ag-specific cells, and to provide greater checks and balances to combat the increased somatic mutation load that longer-lived animals necessarily carry.

Summary

While it is hard to draw global conclusions about the significance of differences between mouse and human immunology, it is worth considering the possibility that any given response in a mouse may not occur in precisely the same way in humans. While caution in interpreting preclinical data obtained in mice is clearly warranted, we believe that with these caveats in mind, mice will continue to be the premiere in vivo model for human immunology and will be absolutely essential for continued progress in our understanding of immune system function in health and disease.

Acknowledgments

Due to space limitations we are unable to cite as many primary references as we would wish and so have instead steered the reader toward many excellent reviews. We offer apologies to those colleagues whose work we were unable to include. We thank the following people for helpful suggestions: Jeffrey Bender, Mark Boothby, Alfred Bothwell, Michael Cahalan, David Camerini, Paolo Casali, George Chandy, Cheong Chang, Nick Crispe, Olja Finn, David Fruman, Geoff Kansas, Mitch Kronenberg, Tom Lane, Klaus Ley, Ruslan Medzhitov, Andre Ouellette, Peter Parham, Howard Petrie, Jordan Pober, Bruce Rosengard, Michael Selsted, Carl Ware, Marian Waterman, and Arthur Weiss. We also thank David Fruman and Craig Walsh for critical reading of the manuscript.

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